Biochem. J. (1986) 237, 807–812 (Printed in Great Britain)

Insulin resistance is accompanied by impairment of amylase-gene expression in the exocrine pancreas of the obese Zucker rat

Elisabeth R. TRIMBLE,* Roberto BRUZZONE* and Dominique BELIN†
*Institut de Biochimie clinique and †Department of Pathology and Institut d'Histologie et d'Embryologie, Centre Médical Universitaire, University of Geneva, 1211 Geneva 4, Switzerland

Insulin plays a major role in the control of pancreatic amylase biosynthesis. In this study we determined glucose metabolism by pancreatic acini as well as the pancreatic content of both amylase protein and amylase mRNA during development of insulin resistance in the obese Zucker rat. At age 4 weeks there were no abnormalities detected in the above parameters, although the obese animals were already hyperinsulinaemic. At 6 weeks glucose metabolism was decreased by 50% in acini from obese rats, whereas pancreatic amylase-gene expression was only slightly impaired. At 22 weeks glucose metabolism was decreased by 50%, amylase content by 55% and amylase mRNA by 60% in acinar tissue of obese rats. As expected, hyperinsulinaemia increased markedly with age. Thus development of severe insulin resistance was associated with impairment of amylase-gene expression. To decrease insulin resistance, one group of adult obese rats was treated with Ciglitazone for 4 weeks. A lowered plasma insulin concentration without alteration of food intake was taken as evidence of decreased insulin resistance. This was associated with normalization of glucose metabolism and a marked increase of both amylase content of pancreatic tissue and amylase mRNA. In conclusion, both the increase of insulin resistance with age and its partial reversal by Ciglitazone treatment appear to modulate pancreatic amylase-gene expression in the obese Zucker rat.

INTRODUCTION

It is now well established that insulin regulates the expression of the amylase gene in the exocrine pancreas. Thus in insulin-deficient states pancreatic amylase (Söling & Unger, 1972; Korc *et al.*, 1981; Dranginis *et al.*, 1984) and amylase mRNA (Korc *et al.*, 1981; Dranginis *et al.*, 1984) are decreased, and can usually be restored by insulin treatment of the diabetic animal. We and others have found that the pancreatic amylase content is decreased in the adult obese Zucker rat (Bruzzone *et al.*, 1984; Schneeman *et al.*, 1983) and in the obese (C57/BL 6J-*ob*/*ob*) mouse (Trimble *et al.*, 1986), two well-characterized animal models of hyperinsulinaemia and insulin resistance. Although the effects of obesity in the exocrine pancreas had not been studied previously, we had suggested that the lower amylase content of the obese animals may have been the result of insulin resistance (Bruzzone *et al.*, 1984). Support for such a possibility comes from the fact that when insulin-deficient rats are rendered insulin-resistant by being fed with a high-fat diet, insulin treatment can no longer restore pancreatic amylase biosynthesis to normal (Bazin & Lavau, 1979). Furthermore, it has been suggested that the cellular basis for insulin resistance under such circumstances is associated with a reduction in glucose metabolism by the acinar tissue (Bazin & Lavau, 1982).

The purpose of the present investigation was to examine in detail the possible link between decreased amylase content and insulin resistance in the exocrine pancreas of the obese Zucker rat. To this end, measurements of pancreatic amylase, amylase mRNA and glucose metabolism in the exocrine pancreas have been made in young Zucker rats during development of obesity and in the adult rat when obesity and insulin resistance are well established. In an attempt to confirm that the abnormalities found were due to insulin resistance, a group of adult obese rats was treated with Ciglitazone {5-[4-(1-methylcyclohexylmethoxy)benzyl]-thiazolidine-2,4-dione}, a substance extremely potent in decreasing resistance to insulin in obese laboratory animals (Chang *et al.*, 1983; Fujita *et al.*, 1983; Kobayashi *et al.*, 1983; Kirsch *et al.*, 1984).

EXPERIMENTAL

Animals

Female Zucker rats, obese (*fa*/*fa*) and homozygous lean (*Fa*/*Fa*), were given by Professor B. Jeanrenaud (University of Geneva). Rats weaned at 3 weeks were studied when 4, 6 or 22 weeks old. From weaning they were maintained on a standard laboratory chow containing (by wt.) 17% protein, 3% lipid, 58.7% carbohydrate, 4.3% cellulose, 5% minerals and 12% water. One group of obese rats was treated with Ciglitazone (80 mg/kg per day). Treatment with Ciglitazone commenced at age 18 weeks and continued for 4 weeks. Body weight, food intake, plasma glucose and plasma insulin concentrations of treated and control groups (obese and lean) were monitored during this period. All animals were caged in a temperature-controlled room (23 °C) with a 12 h-dark/12 h-light cycle.

Animals were killed by decapitation in the fed state between 08:00 and 10:00 h. The pancreas was rapidly removed and a small portion of the splenic part was homogenized before determinations of protein and amylase content (Trimble *et al.*, 1985). The remainder of the pancreas was then taken either for preparation of dispersed acini and measurement of glucose metabolism or for RNA extraction.

Preparation of dispersed acini and measurement of glucose metabolism in pancreatic acini

Dispersed acini were prepared by collagenase digestion as described by Bruzzone *et al.* (1985). Acini were suspended in KRB–Hepes–HSA (12.5 mM-Hepes, 135 mM-NaCl, 4.8 mM-KCl, 1.0 mM-$CaCl_2$, 1.2 mM-KH_2PO_4, 1.2 mM-$MgSO_4$, 5.0 mM-$NaHCO_3$, 300–600 kallikrein-inhibitory units of aprotinin/ml, 0.1% human serum albumin) containing 1 mM-glucose and sufficient (2 μl) anti-insulin serum to bind all insulin released from the few remaining β-cells (the amount was determined in pilot experiments). After 30 min preincubation, during which the tissue was continuously gassed with O_2/CO_2 (19:1) the acini were washed by repeated rinsing and sedimentation to remove all antiserum.

For glucose metabolism, duplicate samples (1 ml) of the acinar suspension were placed in scintillation vials with 1 ml of KRB–Hepes–HSA containing 1 mM-glucose, 1.2 μCi of D-[U-^{14}C]glucose, and various concentrations of insulin (0–1 μM). The vials were closed with a stopper equipped with a centre well and gassed with O_2/CO_2 (19:1) for 1 min. The acini were incubated at 37 °C for 60 min and the reaction was stopped by injection of 0.5 ml of 3 M-H_2SO_4 through the cap into the incubation medium. Liberated CO_2 was trapped by 0.3 ml of 1 M-Hyamine hydroxide injected into the centre well: the CO_2 was collected during 90 min while the vials were kept at 37 °C. Samples of the original acinar preparation were stored for DNA measurements.

RNA extraction and analysis

Pancreases were rapidly placed in guanidine thiocyanate (10 ml per pancreas) and total RNA was isolated essentially as described by Schibler *et al.* (1980). RNA was denatured with glyoxal, electrophoresed in 1.4%-agarose gels and transferred to Biodyne A nylon membranes (Pall) as described by Thomas (1980). For dot-blot hybridization the RNAs were spotted on nitrocellulose (Schleicher and Schuell, BA85) as described by White & Bancroft (1982). Filters were hybridized at 58 °C with 15 ng of heat-denatured plasmid pMPa 21 DNA/ml, a mouse pancreatic α-amylase cDNA probe (Schibler *et al.*, 1980), nick-translated to a specific radioactivity of 0.4×10^8–1.3×10^8 c.p.m./μg (Belin *et al.*, 1984). After 36 h the filters were washed twice at 58 °C with 3 × SSC (1 × SSC = 0.15 M-NaCl/0.015 M-sodium citrate, pH 7.0)/2 × Denhardt's solution, and four times at 35 °C with 0.1 × SSC/0.1% SDS/0.1% $Na_4P_2O_7$. The filters were exposed to Kodak XAR-5 films at −80 °C between Dupont Cronex ParSpeed intensifying screens. The spots from the dot-blots were cut and counted for radioactivity (three times for 10 min) to compare α-amylase mRNA content. The RNAs were translated *in vitro* by using wheat-germ extracts (Dobberstein & Blobel, 1977). The translation products were subjected to SDS/polyacrylamide-gel electrophoresis (Laemmli, 1970) and revealed by fluorography. The migration of standard proteins (Pharmacia low-M_r kit) was determined by Coomassie Blue staining.

Assays

Glucose was measured by the glucose oxidase technique (Huggett & Nixon, 1957), and insulin by radioimmunoassay (Trimble *et al.*, 1982). Protein was measured by the method of Bradford (1976). Amylase was measured by a modification of the method of Bernfeld (1955), with soluble starch as substrate and maltose monohydrate as standard. DNA was measured with diaminobenzoic acid (Beckmann *et al.*, 1981).

Materials

Chemicals were obtained as follows: collagenase (425 'Mandl-units'/mg), Serva, Heidelberg, Germany; D-[U-^{14}C]glucose (346 Ci/mol), New England Nuclear, Dreieich, Germany; Ciglitazone, Takeda Chemical Industries, Osaka, Japan; aprotinin, Bayer, Wuppertal, Germany.

Statistics

Student's unpaired *t* test was used. Results are expressed as means ± S.E.M. or means + range.

RESULTS

The body-weight development of lean and obese rats is shown in Table 1. The plasma glucose concentrations of lean and obese animals were similar at all ages, but, as expected, obese rats were always hyperinsulinaemic with respect to lean controls, and this hyperinsulinaemia became more marked with age (Table 1). This indicates increasing insulin resistance with increasing age.

The rate of glucose metabolism was similar in isolated acini from 4-week-old lean and obese rats. By contrast, at both 6 and 22 weeks, acini from obese rats metabolized much less glucose than did those from lean controls (Table 1). Insulin (1 pM, 1 nM and 1 μM) had no acute effect on glucose metabolism when added during the 1 h incubation period of acini of lean or obese rats (results not shown).

Amylase content was similar in obese and lean animals at 4 and 6 weeks. However, at 22 weeks the amylase content of obese animals was only 45% of that of lean controls (Fig. 1*a*). By contrast, at all ages, total pancreatic protein and pancreatic weight were similar for obese and lean animals (Table 1).

The relative content of amylase mRNA in total RNA for each age of rat is shown in Fig. 1(*b*). At 4 weeks there was no difference between lean and obese rats. However, at 22 weeks the amylase mRNA of obese rats was decreased to a similar extent to amylase content, and was approx. 40% of that in lean controls. Since insulin resistance as reflected by hyperinsulinaemia became more marked with age (see Table 1), the possibility that the changes in the contents of amylase and amylase mRNA could be due to insulin resistance at the level of the exocrine pancreas was explored in further experiments. Use was made of Ciglitazone, which has proven qualities of decreasing insulin resistance in obese laboratory animals. A group of 14 adult obese animals was treated with this substance for 4 weeks (see the Experimental section) and compared with 13 untreated obese rats. Ciglitazone treatment did not affect food intake (24.5 ± 1.1 and 21.8 ± 0.9 g/day for treated and untreated obese respectively) or weight gain (41.9 ± 3.8 and 44.6 ± 2.1 g/4 weeks for treated and untreated respectively), but caused a marked decrease in plasma insulin levels (5.0 ± 0.6 and 14.6 ± 2.0 ng/ml, in treated and untreated respectively). This was accompanied by a slight fall in plasma glucose concentrations (112 ± 3 to 104 ± 2 mg/dl). These results indicate that Ciglitazone-treated obese rats were more sensitive to insulin than

Table 1. Body weight, plasma glucose, plasma insulin, pancreatic weight, pancreatic protein and acinar glucose metabolism of lean and obese Zucker rats

Glucose metabolism was measured in dispersed pancreatic acini by determining the production of $^{14}CO_2$ from D-[U-^{14}C]glucose. Results (means ± S.E.M.) are expressed as pmol of CO_2/60 min per μg of DNA. *$P < 0.02$, **$P < 0.001$ versus lean.

Rats		n	Body wt. (g)	Plasma glucose (mg/dl)	Plasma insulin (ng/ml)	Pancreatic wt. (mg)	Pancreatic protein (mg/g of pancreas)	Glucose metabolism (pmol of CO_2/60 min per μg of DNA)	n
4 weeks	lean	13	43±3	141±8	0.70±0.13	260±21	167±10	819±165	4
	obese	13	58±4*	148±9	2.77±0.43**	290±23	168±10	745±93	4
6 weeks	lean	9	97±5	146±6	0.58±0.08	547±34	129±18	549±63	5
	obese	15	107±14	153±3	3.73±0.92*	526±20	128±8	275±55*	5
22 weeks	lean	13	208±3	115±3	1.81±0.31	998±23	114±4	767±151	6
	obese	13	400±4**	112±3	14.6±2.00**	953±25	110±4	309±45*	6

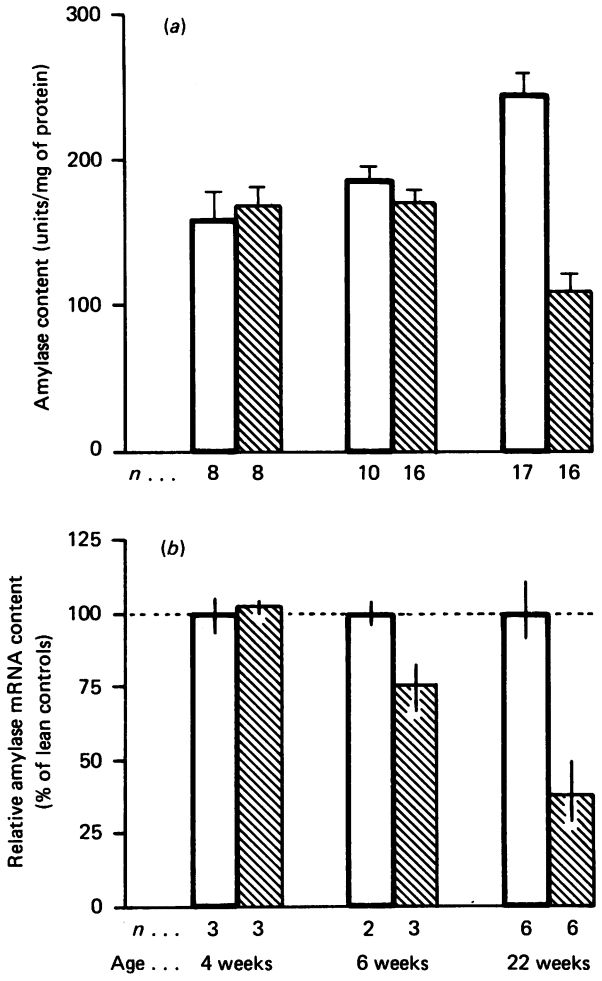

Fig. 1. (*a*) Amylase content and (*b*) relative content of α-amylase mRNA in pancreases of lean and obese Zucker rats

In (*a*) Results are expressed as means ± S.E.M. At 22 weeks the amylase content of pancreases from obese Zucker rats was less than that from lean controls ($P < 0.001$). In (*b*) the relative amylase mRNA content was calculated from dot-blots of total pancreatic RNA hybridized to nick-translated pMPa 21 DNA. For every amount of RNA spotted (156 ng–10 μg in 2-fold serial dilutions; 4–8 dilutions per pancreas), the average hybridized radioactivity (c.p.m.) of the lean controls was taken as 100%. The relative amylase mRNA content for each pancreas was calculated at each dilution. For each experimental group the mean values plus range are indicated. At 22 weeks amylase mRNA was significantly less in obese than lean rats ($P < 0.001$). □, Lean; ▨, obese.

were untreated obese animals. At the same time, Ciglitazone treatment was accompanied by normalization of acinar glucose metabolism, and amylase content was restored to 80% of lean controls (Fig. 2). Similarly, this treatment was accompanied by a marked increase in amylase mRNA content compared with that of untreated obese rats (Fig. 3). Dot-blot analysis showed that the relative amount of amylase mRNA was raised to 60% of that of lean controls ($P < 0.05$ versus untreated obese). A control experiment had shown that Ciglitazone, at the same dose, had no effect on glucose metabolism or amylase content of lean animals (results not shown).

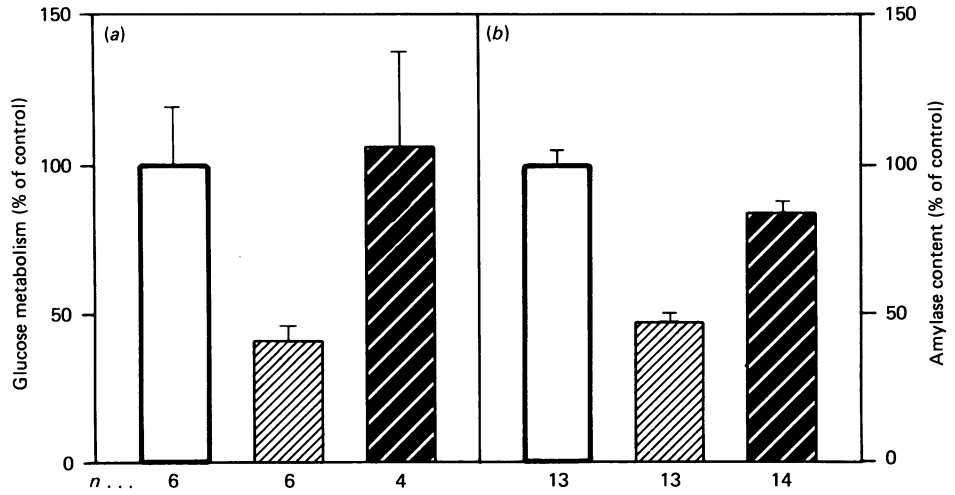

Fig. 2. Effect of Ciglitazone treatment on (*a*) glucose metabolism and (*b*) amylase content in the pancreas of 22-week obese Zucker rats

Obese rats received Ciglitazone (80 mg/kg per day) for 4 weeks. Results are expressed as percentages of values for lean controls (mean ± S.E.M.). Treatment increased glucose metabolism in obese ($P < 0.02$ versus obese untreated) to the same value as in lean controls. Treatment also increased pancreatic amylase content ($P < 0.001$ versus obese untreated), but this remained slightly lower than in lean controls ($P < 0.025$). □, Lean; ▨, obese; ◪, obese treated.

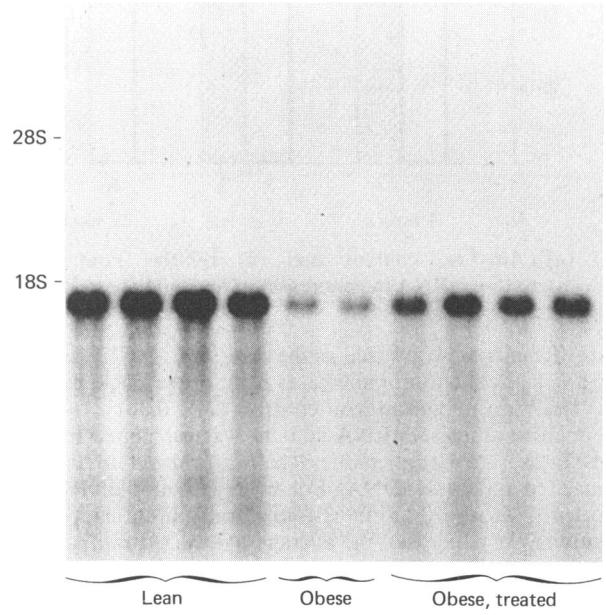

Fig. 3. α-Amylase mRNA content in pancreases of lean, obese, and obese Ciglitazone-treated rats

Total pancreatic RNAs (5 μg/lane) were electrophoresed in a 1.4%-agarose gel and transferred to nitrocellulose. RNAs electrophoresed in adjacent lanes were stained with ethidium bromide to determine the position of 18S and 28S rRNAs. The filter was hybridized to nick-translated pMPa 21 DNA and autoradiographed.

Total pancreatic RNAs from lean and obese animals were translated with similar efficiency in cell-free extracts. The electrophoretic profiles of the translation products showed differences in the relative amount of pre-amylase, which was decreased with RNA from pancreases from obese adults and partially normalized in obese rats treated with Ciglitazone (Fig. 4).

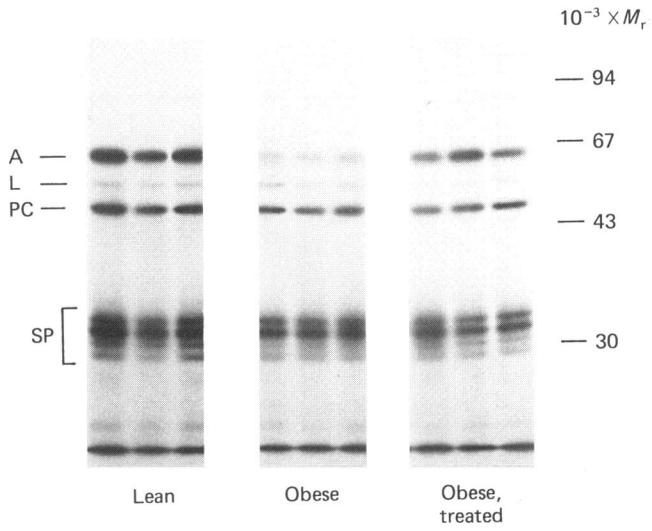

Fig. 4. Profile of pancreatic-RNA translation products

Pancreatic RNAs (5 μg) from lean, obese, and obese Ciglitazone-treated animals (22 weeks) were translated in the presence of [^{35}S]methionine in a wheat-germ extract. The positions of pre-amylase (A), pre-lipase (L), pre-procarboxypeptidase (PC) and serine-proteinase zymogens (SP) are indicated (Giorgi *et al.*, 1984). The M_r values of standard proteins are shown. Three separate animals were used for each condition; individual results are shown.

DISCUSSION

We and others have reported that amylase content is decreased in adult obese Zucker rats (Bruzzone *et al.*, 1984; Schneeman *et al.*, 1983), an abnormality which has also been found recently in the obese (C57 BL/6J-*ob*/*ob*) mouse (Trimble *et al.*, 1986). However, from a decreased content alone one could not deduce whether the biosynthetic rate is decreased or the secretory rate increased. We have shown that secretion of pancreatic

enzymes is decreased in obese Zucker rats both *in vivo* (Bruzzone *et al.*, 1984) and *in vitro* (Trimble & Bruzzone, 1985), and therefore those secretory abnormalities that exist cannot account for the decreased enzyme content. The present study shows that the steady-state amounts of pancreatic amylase mRNA are markedly decreased in adult obese Zucker rats with respect to lean controls. This may result either from a decrease in pancreatic amylase-gene expression at the transcriptional level or from a decreased stability of amylase mRNA in obese rats, which leads to a decrease in the biosynthetic rate and thus to a lower pancreatic amylase content in these animals.

The study of the regulation of pancreatic enzyme content has revealed that it is largely under the control of dietary and hormonal factors. Both the quality and, in extreme cases, the quantity of the diet are important in determining pancreatic enzyme composition (Grosman *et al.*, 1942; Ben Abdeljlil & Desnuelle, 1964; Danielsson *et al.*, 1974; Lee *et al.*, 1982; Saraux *et al.*, 1982; Giorgi *et al.*, 1984; Wicker *et al.*, 1984; Schick *et al.*, 1984*b*). However, since we have previously shown that the hyperphagia of the obese Zucker rat is not responsible for the lower pancreatic amylase content (Bruzzone *et al.*, 1984), dietary factors cannot explain the difference in amylase content between the obese and lean adult Zucker rats in the present study. Several hormonal factors such as glucocorticoids, cholecystokinin and insulin have been implicated in the regulation of pancreatic amylase biosynthesis. Thus glucocorticoid treatment increases (Rall *et al.*, 1977; Harding *et al.*, 1978; Logsdon *et al.*, 1985), whereas infusion with a cholecytoskinin analogue decreases (Schick *et al.*, 1984*a*), the specific activity of pancreatic amylase. Since in the obese Zucker rat there exist both an increase in the corticosterone turnover rate (Cunningham *et al.*, 1986) and a decrease in pancreatic responsiveness to cholecystokinin and its analogues (McLaughlin *et al.*, 1984; Trimble & Bruzzone, 1985), these groups of hormones cannot be implicated as aetiological agents in the decreased specific activity of amylase in the obese Zucker rat.

In view of the paramount role played by insulin in amylase biosynthesis, the development of insulin resistance appeared a more likely cause of decreased contents of amylase and amylase mRNA in the obese rat. Hyperinsulinaemia was already present in 4-week-old obese rats. Hence it is clear that insulin resistance starts at a very early age. Since altered glucose metabolism is often associated with insulin resistance, it was interesting to find that a decrease in glucose oxidation in isolated acini was not seen before 6 weeks of age in obese rats. This decrease was similar to that reported by others (Bazin & Lavau, 1982) in rats made insulin-resistant by a high fat diet. As observed in that study (Bazin & Lavau, 1982), insulin did not have an acute effect on glucose oxidation. It is important to remember that insulin resistance is known to develop at very different times in the various insulin-dependent pathways in the obese Zucker rat (Crettaz *et al.*, 1980) and in other forms of obesity (Le Marchand-Brustel *et al.*, 1978). Thus the age at which decreases in amylase content first arise (Bruzzone *et al.*, 1984) in no way precludes a possible aetiological link with insulin resistance.

In an attempt to establish a closer relationship between insulin resistance and the decreased pancreatic amylase content of obese rats we employed the substance Ciglitazone. Its effects have been studied by Fujita *et al.* (1983) in obese insulin-resistant animals, with little or no effect being demonstrable in normal or insulinopenic rats. Those authors showed that Ciglitazone treatment of the obese Zucker rat results in enhanced insulin sensitivity *in vivo*. We showed in the present study that, in obese Zucker rats, Ciglitazone therapy was associated with a fall in plasma insulin concentrations without a change in food intake. Concomitantly, normalization of glucose oxidation and near-normalization of amylase and amylase mRNA contents occurred in the exocrine pancreas. Thus treatment that resulted in a decrease in insulin resistance in the adult obese rat was associated with an increase in amylase protein and amylase mRNA.

It therefore becomes clear that insulin resistance has an impact on the function of the exocrine pancreas in addition to the more classically studied insulin-responsive tissues, such as muscle, liver and adipose tissue. The present study strongly suggests that insulin resistance is associated with a decrease in pancreatic amylase-gene expression.

We acknowledge with gratitude the following gifts: plasmid pMPa 21 from Dr. Ueli Schibler, University of Geneva, and Ciglitazone from Dr. M. Nishikawa, Takeda Chemical Industries, Osaka, Japan. We thank Dr. J.-D. Vassalli for helpful discussion and Dr. Guy Cuendet for pelletting of Ciglitazone. The expert technical assistance of Ms. N. Challet, Ms. C. Combépine and Ms T. Cuche, and animal care by Mr. C. Jorand, are also acknowledged. The investigation was supported by grants from the Fonds National Suisse de la Recherche Scientifique nos. 3.159-0.81 and 3.246-0.82 SR.

REFERENCES

Bazin, R. & Lavau, M. (1979) Digestion **19**, 386–391
Bazin, R. & Lavau, M. (1982) Am. J. Physiol. **243**, G448–G454
Beckmann, J., Holze, S., Lenzen, S. & Panten, U. (1981) Acta Diabetol. Lat. **18**, 51–57
Belin, D., Godeau, F. & Vassalli, J.-D. (1984) EMBO J. **3**, 1901–1906
Ben Abdeljlil, A. & Desnuelle, P. (1964) Biochim. Biophys. Acta **81**, 136–149
Bernfeld, P. (1955) Methods Enzymol. **1**, 149–158
Bradford, M. M. (1976) Anal. Biochem. **72**, 248–254
Bruzzone, R., Trimble, E. R., Gjinovci, A. & Renold, A. E. (1984) Biochem. J. **219**, 333–336
Bruzzone, R., Halban, P. A., Gjinovci, A. & Trimble, E. R. (1985) Biochem. J. **226**, 621–624
Chang, A. Y., Wyse, B. M. & Gilchrist, B. J. (1983) Diabetes **32**, 839–845
Crettaz, M., Prentki, M., Zaninetti, D. & Jeanrenaud, B. (1980) Biochem. J. **196**, 525–534
Cunningham, J. J., Calles-Escandon, J., Garrido, F., Carr, D. B. & Bode, H. H. (1986) Endocrinology (Baltimore) **118**, 98–101
Danielsson, A., Marklund, S. & Stigbrand, T. (1974) Acta Hepato-Gastroenterol. **21**, 289–297
Dobberstein, B. & Blobel, G. (1977) Biochem. Biophys. Res. Commun. **74**, 1675–1682
Dranginis, A., Morley, M., Nesbitt, M., Rosenblum, B. B. & Meisler, M. H. (1984) J. Biol. Chem. **259**, 12216–12219
Fujita, T., Sugiyama, Y., Taketomi, S., Sohda, T., Kawamatsu, Y., Iwatsuka, H. & Suzouki, Z. (1983) Diabetes **32**, 804–810
Giorgi, D., Bernard, J. P., Lapointe, R. & Dagorn, J. C. (1984) EMBO J. **3**, 1521–1524
Grossman, M. I., Greengard, H. & Ivy, A. C. (1942) Am. J. Physiol. **138**, 676–682

Harding, J. D., Przybyla, A. E., MacDonald, R. J., Pictet, R. L. & Rutter, W. J. (1978) J. Biol. Chem. **253**, 7531–7537
Huggett, A. St. G. & Nixon, D. A. (1957) Biochem. J. **66**, 12P
Kirsch, D. M., Bachmann, W. & Häring, H. U. (1984) FEBS Lett. **176**, 49–54
Kobayashi, M., Iwasaki, M., Ohgaku, S., Maegawa, H., Watanabe, N. & Shigeta, Y. (1983) FEBS Lett. **163**, 50–53
Korc, M., Owerbach, D., Quinto, C. & Rutter, W. J. (1981) Science **213**, 351–353
Laemmli, U. K. (1970) Nature (London) **227**, 680–685
Lee, P. C., Brooks, S. & Lebenthal, E. (1982) Am. J. Physiol. **242**, G215–G221
Le Marchand-Brustel, Y., Freychet, P. & Jeanrenaud, B. (1978) Endocrinology (Baltimore) **102**, 74–85
Logsdon, C. G., Moessner, J., Williams, J. A. & Goldfine, I. D. (1985) J. Cell Biol. **100**, 1200–1208
McLaughlin, C. L., Peikin, S. R. & Baile, C. A. (1984) Physiol. Behav. **32**, 961–965
Rall, L., Pictet, R., Githens, S. & Rutter, W. J. (1977) J. Cell Biol. **75**, 398–409
Saraux, B., Girard-Globa, A., Ouagued, M. & Vacher, D. (1982) Am. J. Physiol. **243**, G10–G15
Schibler, U., Tosi, M., Pittet, A.-C., Fabiani, L. & Wellauer, P. K. (1980) J. Mol. Biol. **142**, 93–116
Schick, J., Kern, H. & Scheele, G. A. (1984*a*) J. Cell Biol. **99**, 1569–1574
Schick, J., Verspohl, R., Kern, H. & Scheele, G. A. (1984*b*) Am. J. Physiol. **247**, G611–G616
Schneeman, B. O., Inman, M. D. & Stern, J. S. (1983) J. Nutr. **113**, 921–925
Söling, H. D. & Unger, K. O. (1972) Eur. J. Clin. Invest. **2**, 199–212
Thomas, P. S. (1980) Proc. Natl. Acad. Sci. U.S.A. **77**, 5201–5205
Trimble, E. R. & Bruzzone, R. (1985) Regul. Pept. **11**, 227–235
Trimble, E. R., Halban, P. A., Wollheim, C. B. & Renold, A. E. (1982) J. Clin. Invest. **69**, 405–413
Trimble, E. R., Bruzzone, R., Gjinovci, A. & Renold, A. E. (1985) Endocrinology (Baltimore) **117**, 1246–1252
Trimble, E. R., Bruzzone, R. & Herberg, L. (1986) Comp. Biochem. Physiol. A **83**, 387–390
White, B. A. & Bancroft, F. C. (1982) J. Biol. Chem. **257**, 8569–8572
Wicker, C., Puigserver, A. & Scheele, G. A. (1984) Eur. J. Biochem. **139**, 381–387

Received 5 September 1985/21 March 1986; accepted 8 April 1986